identified by a method comprising: screening a random peptide library of peptides of about 10 to 100 amino acids with a ligand, said ligand being a peptide of 36 amino acids or fewer, in which the ligand is an epitope of an antigen that is specifically bound by said antibody or in which the ligand represents the portion of a receptor-ligand that is responsible for the specific binding of the receptor to the receptor-ligand.

45. (amended) The compound of claim 2 in which the antibody or antigen-binding derivative thereof specifically binds to a human tumor antigen.

Please add the following new claims:

47. (new) The compound of claim 2 or 8 wherein the peptide is about 20 to 50 amino acids and the second random peptide library is of peptides of about 20 to 50 amino acids.

48. (new) The compound of claim 36 wherein the peptide is about 20 to 50 amino acids and the random peptide library is of peptides of about 20 to 50 amino acids.

REMARKS

Prior to the above-made amendments, claims 2 to 4, 6, 8, 10, 13, 15 to 17, 19, 21, 23, 24, 27, 30, 33, 35, 36, 40, and 43 to 46 were pending in the instant application. Claims 13, 15 to 17, 19, 21, 23, 24, 33, 35, 40, 43, 44, and 46 are withdrawn from consideration as being directed to a non-elected invention and accordingly, have been canceled without prejudice to Applicant's right to pursue the subject matter of the canceled claims in a continuation or divisional patent application. Claims 2 to 4, 6, 8, 10, 27, 30, 36, and 45 have been clarified and claims 47 and 48 have been added to more particularly point out and distinctly claim that which Applicant deems to be the subject of the invention. With entry of the above-made amendments, claims 2 to 4, 6, 8, 10, 27, 30, 36, 45, 47, and 48 will be pending. A marked up version of the claims showing the amendments made is attached as Exhibit A and a copy of the claims that will be pending upon entry of the present amendments is attached as Exhibit B.

Claims 2 to 4, 6, 8, 10, 27, 30, 36, and 45 have been amended to replace the recitation of "molecule" with "compound". Support is found in the specification as originally filed on page 92, lines 17 to 19.

Claims 2, 6, and 36 have been amended to recite that the peptide which mimics the binding specificity of an antibody is about 10 to 100 amino acids which is identified by screening a peptide library of peptides of about 10 to 100 amino acids. Support is found in the specification as originally filed on page 23, lines 3 to 4.

Claim 45 has been amended to recite that the antibody or antigen-binding derivative thereof specifically binds to a human tumor antigen. Support is found in the specification as originally filed on page 26, lines 13 to 15 and page 70, lines 30 to 35.

Claims 47 and 48 have been added to recite that the peptide which mimics the binding specificity of an antibody is about 20 to 50 amino acids which is identified by screening a peptide library of peptides of about 20 to 50 amino acids. Support is found in the specification as originally filed on page 23, lines 4 to 5.

Applicant submits that the above-made amendments are fully supported in the instant application as originally filed, and do not constitute new matter. Applicant respectfully requests that the above-made amendments be entered into the file history of the instant application.

THE REJECTION UNDER 35 U.S.C. § 101, SHOULD BE WITHDRAWN

Claims 2 to 4, 6, 8, 10, 27, 30, 36, and 45 are rejected under 35 U.S.C. § 101 because the claimed invention is alleged to be directed to non-statutory subject matter. The Examiner asserts that a "molecule" is non-statutory subject matter and that amending to claim a "compound" will overcome this rejection.

In response, Applicant respectfully points out that claims 2 to 4, 6, 8, 10, 27, 30, 36, and 45 have been amended to recite a "compound" in place of a "molecule". Thus, Applicant submits that the rejection has been obviated and respectfully requests withdrawal of the rejection.

THE REJECTION UNDER 35 U.S.C. § 112, SECOND PARAGRAPH, SHOULD BE WITHDRAWN

Claim 45 is rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner contends that the term "is capable of binding to a human tumor antigen" is not definite as to whether the "antibody or antigen-binding derivative thereof" must currently bind a human tumor antigen or can somehow derivatized to be made capable of binding such antigen.

In response, Applicant respectfully points out that claim 45 has been amended to recite that the antibody or antigen-binding derivative thereof specifically binds to a human tumor antigen. Thus, Applicant submits that the rejection has been obviated and respectfully requests withdrawal of the rejection.

THE REJECTION UNDER 35 U.S.C. § 102(a) OR § 102(b) OR ALTERNATIVELY UNDER 35 U.S.C. § 103(a), SHOULD BE WITHDRAWN

Claims 2 to 4, 6, 8, 10, 27, 30, 36 and 45 are rejected under 35 U.S.C. § 102(a) or 102(b) as anticipated by or, in the alternative, under 35 U.S.C. § 103(a) as obvious over Griffiths *et al.*, WO 93/11236 (6/93), hereinafter "Griffiths".

The Examiner alleges that Griffiths discloses peptides which are "anti-self antibody fragments" which bind "self antigens" and that the Griffith antibody peptide fragments are "molecules comprising a peptide which mimics the binding specificity of an antibody" since the fragments mimic the ability of the parent antibody to bind the same antigen.

Applicant respectfully disagrees with these rejections and with the Examiner's characterization of Griffiths.

THE LEGAL STANDARD

In order for a reference to anticipate a claim, each and every element of the claim must be disclosed in that one reference. Orthokinetics, Inc. v. Safety Travel Chairs, Inc., 1 U.S.P.Q.2d 1081 (Fed. Cir. 1985). "Anticipation under Section 102 can be found only if a reference shows exactly what is claimed. . . ." Structural Rubber Prod. Co. v. Park

Rubber Co., U.S.P.Q. 1264 (Fed. Cir. 1984).

The objective standard for obviousness under 35 U.S.C. § 103 as set forth clearly by the Supreme Court of the United States in Graham v. John Deere Co., 383 U.S. 1 (1966) requires the Examiner to ascertain: (1) the scope and content of the prior art; (2) the level of ordinary skill in the art; and (3) the differences between the claimed subject matter and the prior art. *See* 383 U.S. at 17. The obviousness or nonobviousness of the claimed subject matter must be determined in light of these inquiries. Moreover, the Graham Court also explained that secondary considerations such as commercial success, long felt but unsolved needs, failure of others, *etc.* might be utilized in determining the obviousness or nonobviousness of the invention.

Following Graham, the Court of Customs and Patent Appeals (CCPA) and its present successor, the Court of Appeals for the Federal Circuit (CAFC), have held the following considerations to be objective evidence of nonobviousness: long felt need, commercial success, failure of others, copying and unexpected results. *See, e.g.,* Avia Group Int'l Inc. v. L.A. Gear California, Inc., 853 F.2d 1557, 7 U.S.P.Q.2d 1548 (Fed. Cir. 1988); In re Sernaker, 702 F.2d 989, 217 U.S.P.Q. 1 (Fed. Cir. 1983). In fact, the CAFC has consistently made clear that when evidence of such secondary considerations is present, it must be considered by the Examiner or a court in determining a question of obviousness. *See e.g.*, Hybritech, Inc. v. Monoclonal Antibodies, Inc., 802 F.2d 1367, 1379-80, 231 U.S.P.Q. 81, 90 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987); Stratoflex Inc. v. Aeroquip Corp., 713 F.2d 1530, 1538-39, 218 U.S.P.Q. 871, 879 (Fed. Cir. 1983).

A rejection for obviousness is improper when there is nothing in the cited prior art references, either singly or in combination, to suggest the desirability of the claimed subject matter.

A finding of obviousness requires that the prior art suggest to those of ordinary skill in the art (1) that they should carry out the invention and (2) that they would have a reasonable expectation of success in so doing.

See In re Vaeck, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991), where the Federal Circuit said:

> [A] proper analysis under §103 requires, *inter alia*, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or

carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. *See In re Dow Chemical Co.,* 837 F2d 469, 473, 5 U.S.P.Q. 2d 1529, 1531 (Fed. Cir. 1988). [emphasis added]

947 F.2d at 493, 20 U.S.P.Q.2d at 1442.

GRIFFITHS DOES NOT ANTICIPATE AND/OR MAKE OBVIOUS THE PRESENTLY CLAIMED INVENTION

The presently claimed invention encompasses compounds comprising peptides identified by a two-step process of screening random peptide libraries. In the first step, the target ligand is an antibody, receptor or derivative thereof. The peptides identified in the first screening step are used as target ligands in the second screening step. The peptides identified in the second screening step are abtides. Abtides possess binding specificities that are similar to the binding specificities of the antibodies or receptors that are used in the first screening step. An abtide is typically a peptide that mimics, with respect to binding specificity, and possibly other characteristics (*e.g.*, binding affinity, sequence, *etc.*) a large molecule such as an antibody or receptor. However, as specifically taught in the specification, *e.g.*, at page 23, line 1, an abtide is generally much smaller than an antibody or receptor. Claims 2, 6, and 38 have been amended to recite a peptide of about 10 to 100 amino acids which mimics the binding specificity of an antibody. Claims 47 and 48 have been added to recite that the peptide which mimics the binding specificity of an antibody is about 20 to 50 amino acids. Thus, as presently claimed, the instant invention encompasses abtides of 10 to 100 amino acids, optionally, 20 to 50 amino acids. The use of abtides has many potential advantages over the use of antibodies: the smaller size of abtides allows their easier production at lower cost, reduced immunogenicity, and may facilitate their *in vivo* delivery if such is desired.

Griffiths, on the other hand, relates to the isolation of antibody molecules directed against self antigens, *e.g.*, human antibodies directed against human self antigens. As described in Griffiths, an antibody fragment is formed with a V_H or V_L domain of an antibody, or any part of an antibody which, either alone or in combination with one or more other component parts. Examples of polypeptide chains which may be used as component parts include, in addition to V_H and V_L domains, V_LC_L, V_HC_H1, scFv fragments, Fab fragments and so on (see, *e.g.*, page 7, lines 21 to 30 of Griffiths). Thus, the antibody fragment described in Griffiths is not a peptide of 10 to 100 amino acids of the presently

claimed invention, but rather, the antibody fragment corresponds to one or more domains, *e.g.*, V_H or V_L and combinations thereof, of an antibody that is capable of binding an epitope or antigen. The smallest antibody fragment described in Griffith if a V_H or V_L domain. As is known to one of skill in the art, the size of a V_H or V_L domain is at least 110 amino acids; this is certainly larger than the peptide of 10 to 100 amino acids of the presently claimed invention.

As evidence that the size of V_H or V_L domain is at least 110 amino acids, Applicant submits concurrently herewith pages 86 to 90 from The Experimental Foundations of Modern Immunology, 3rd Edition, 1986, published John Wiley & Sons, New York as Exhibit C and pages 38 to 46 from Cellular and Molecular Immunology, 2nd Edition, 1994, published W.B. Saunders Company, Philadelphia as Exhibit D. The size of the V_H and V_L regions is described as about 110 amino acids (see, *e.g.*, second full paragraph, second sentence on page 86 of Exhibit C, right column, third sentence on page 38 of Exhibit D, left column, first sentence on page 43 of Exhibit D) and at least 110 amino acids (see, *e.g.*, Figure 4-12 on page 89 of Exhibit C and Figure 3-4 on page 42 of Exhibit D).

The other antibody fragments described in Griffith, *i.e.*, V_LC_L, V_HC_H1, scFv fragments, Fab fragments, are combinations of V_H or V_L domains. For example, a scFv fragment is a V_H domain joined to a V_L domain by a polypeptide linker (see, *e.g.*, page 10, lines 23 to 28 of Griffith). A Fab fragment results from proteolytic cleavage of an IgG molecule and contains V_H, C_H1, V_L, and C_L domains (see, *e.g.*, Figure 3-8 on page 46 of Exhibit D). Thus, all of the antibody fragments described above contain at least a V_H or V_L domain, each of which is at least 110 amino acids. Hence, the minimum size of any antibody fragment described in Griffiths is 110 amino acids. Thus, Applicant respectfully submits that the smallest antibody fragment described by Griffith is a V_H or V_L domain which is at least 110 amino acids long, which is larger than the 10 to 100 amino acid peptide of the presently claimed invention.

Therefore, the antibody fragments described in Griffiths are not the same as abtides. As described in In re Thorpe, 777 F.2d 695, 698, 227 U.S.P.Q. 964, 966 (Fed.Cir. 1985), "the prior art products do not necessarily or inherently possess the characteristics of his claimed product" since antibody fragments and abtides are distinct products.

Prior to the present invention, there was no reasonable expectation that the

binding of antibodies could be mimicked by much smaller molecules such as the abtides from the libraries described in the present application without the use of scaffolds to hold the abtides in proper configuration. When the prior art sought to mimic the binding of antibodies using peptide libraries, this was generally done by expressing naturally occurring antibody sequences, *e.g.*, entire variable region genes, cloned into phage or phagemid expression vectors. Implicit in the use of such large binding regions to mimic antibodies was the belief that relatively small peptides without defined structure, such as abtides, would not suffice to mimic the binding specificity of the much larger antibodies.

The antibody fragments of Griffiths are distinct from the peptides of the presently claimed invention for all of the reasons described above. Specifically, antibody fragments are not peptides of about 10 to 100 amino acids that mimic the binding specificity of an antibody, but rather, are domains of an antibody of at least 110 amino acids that bind to an antigen of interest. As described in the specification on page 10, lines 10 to 17, the advantages of abtides include smaller size and reduced immunogenicity.

Accordingly, Griffiths does not describe, suggest, nor provide a reasonable expectation of success of the presently claimed invention of molecules comprising peptides of about 10 to 100 amino acids that mimic the binding specificity of an antibody and compositions. Therefore, the above remarks and amendments overcome or obviate the rejections under § 102 and/or §103, and their withdrawal is requested.

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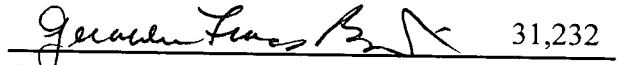
CONCLUSION

Applicant respectfully requests entry of the foregoing amendments and remarks into the file history of the above-identified application. Applicant believes that each ground for rejection or objection has been successfully overcome or obviated, and that all the pending claims are in condition for allowance. Withdrawal of the Examiner's rejections and objections, and allowance of the application are respectfully requested.

Respectfully submitted,

Date January 14, 2003



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Enclosures

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EXHIBIT A: MARKED UP VERSION OF THE CLAIMS

(U.S. APPLICATION NO. 09/484,879; ATTORNEY DOCKET NO. 1101-226)

2. (twice amended) A [molecule] compound comprising a peptide of about 10 to 100 amino acids which mimics the binding specificity of an antibody, which peptide is identified by a method comprising:

(a) screening a first random peptide library with an antibody or antigen-binding derivative thereof that specifically binds to an antigen of interest, to identify a first peptide that specifically binds to said antibody or antigen-binding derivative thereof; and

(b) screening a second random peptide library of peptides of about 10 to 100 amino acids which is the same or different from said first random peptide library with a compound comprising said first peptide identified in step (a) or a specific binding portion thereof, to identify a second peptide which binds to said compound and which mimics the binding specificity of said antibody.

3. (amended) The [molecule] compound of claim 2, in which said first random peptide library is a different library from said second random peptide library.

4. (amended) The [molecule] compound of claim 2, in which said first random peptide library is the same library as said second random peptide library.

6. (twice amended) A [molecule] compound comprising a peptide of about 10 to 100 amino acids which mimics the binding specificity of an antibody, which peptide is identified by a method comprising:

(a) screening a first random peptide library with an antibody or antigen-binding derivative thereof, to identify a plurality of different first peptides each of which specifically binds to said

antibody or antigen-binding derivative thereof;

(b) comparing the sequences of said plurality of different first peptides identified as binding said antibody or antigen-binding derivative thereof in step (a), to identify a consensus binding sequence; and

(c) screening a second random peptide library of peptides of about 10 to 100 amino acids which is the same or different from said first random peptide library with a compound comprising said consensus binding sequence, to identify a second peptide which binds to said compound and which mimics the binding specificity of said antibody.

8. (twice amended) The [molecule] compound of claim 2 in which the antibody is the monoclonal antibody 7E11-C5 which is a murine IgG1 monoclonal antibody which binds specifically to human prostate carcinoma cell line LNCaP, as produced by the hybridoma deposited with the ATCC and assigned accession number HB 10494.

10. (twice amended) The [molecule] compound of claim 2 in which the library of step (a) or step (b) is a library of recombinant vectors that express a plurality of heterofunctional fusion proteins comprising random peptides, said fusion proteins comprising a binding domain encoded by an oligonucleotide comprising unpredictable nucleotides in which the unpredictable nucleotides are arranged in one or more contiguous sequences, wherein the total number of unpredictable nucleotides is greater than or equal to about 15 and less than or equal to about 600, and an effector domain that enhances expression or detection of the binding domain.

27. (twice amended) A composition comprising the [molecule] compound of claim 2; and a carrier.

30. (twice amended) A composition comprising the [molecule] compound of claim 8; and a pharmaceutically acceptable carrier.

36. (twice amended) A [molecule] compound comprising a peptide of about 10 to 100 amino acids which mimics the binding specificity of an antibody, which peptide is identified by a method comprising: screening a random peptide library of peptides of about 10 to 100 amino acids with a ligand, said ligand being a peptide of 36 amino acids or fewer, in which the ligand is an epitope of an antigen that is specifically bound by said antibody or in which the ligand represents the portion of a receptor-ligand that is responsible for the specific binding of the receptor to the receptor-ligand.

45. (amended) The [molecule] compound of claim 2 in which the antibody or antigen-binding derivative thereof [is capable of] specifically [binding] binds to a human tumor antigen.

THE EXPERIMENTAL FOUNDATIONS OF MODERN IMMUNOLOGY

THIRD EDITION

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animals, and more rarely in animals of two different species. Especially in the latter case, cross-reactivity may be fortuitous, reflecting the existence of similar but not identical V regions that have been elicited in response to the same antigen.

Immunoglobulin Fine Structure

The Concept of Domains

As the primary structure of H and L chains began to be worked out through amino acid sequencing of myeloma proteins, certain features suggesting linear repeats of homologous polypeptide units became apparent. The first of these features to be discerned was the regular spacing of half-cystine residues along the H and L chains. In the native globular state, these residues, about 60 amino acids apart, are known to interact to form intrachain disulfide bonds, with resulting "loops" in the Ig chains (Figure 3-5). In human κ or λ chains, for example, there is a total of four half-cystine residues involved in intrachain disulfide bonding, resulting in one loop each in the variable and constant region. In H chains, there is one such loop in the V_H region, and a total of three or four (depending on the Ig class) loops regularly spaced in the C_H portion of the chain. The V_L and C_L domains line up with the V_H and first C_H domains, and the remaining C_H domains of the two H chains also line up with one another.

This periodicity of intrachain disulfide bonds led to a closer examination of the amino acid sequences in the C region of H chains; such studies, together with subsequent X-ray crystallographic data (Chapter 6), have confirmed the notion that Ig peptides are composed of consecutive, related sequences of about 110 amino acids ("homology units"). The V_H and V_L regions are, of course, heterogeneous in structure, but their size of about 110 amino acids suggests they they too bear some relation to the basic repeat unit. It is generally thought that the present Ig-chain structure arose by duplication of a primordial structural gene responsible for an approximately 110-amino acid peptide. At some early point, the gene replicate coding for the primitive V region is thought to have split off and come under separate genetic regulation, the various C regions then evolving by a process of gene replication and segregation. In the case of L chains, one V_L and one C_L unit, or "domain," became joined together to form the functional peptide structure. For H chains, the primoridal C_H gene is thought to have undergone additional replication to form a more complex gene coding for three or four domains, which are joined at some point with one V_H domain to complete the heavy chain unit peptide (Figure 4-11). We will see in Chapter 5 that at the molecular level, the DNA sequences coding for C_H region domains are in fact physically separate from one another (although grouped closely together), supporting the idea of coevolution from a domain-sized primitive gene.

The regions within the disulfide loop of each domain are rather compact,

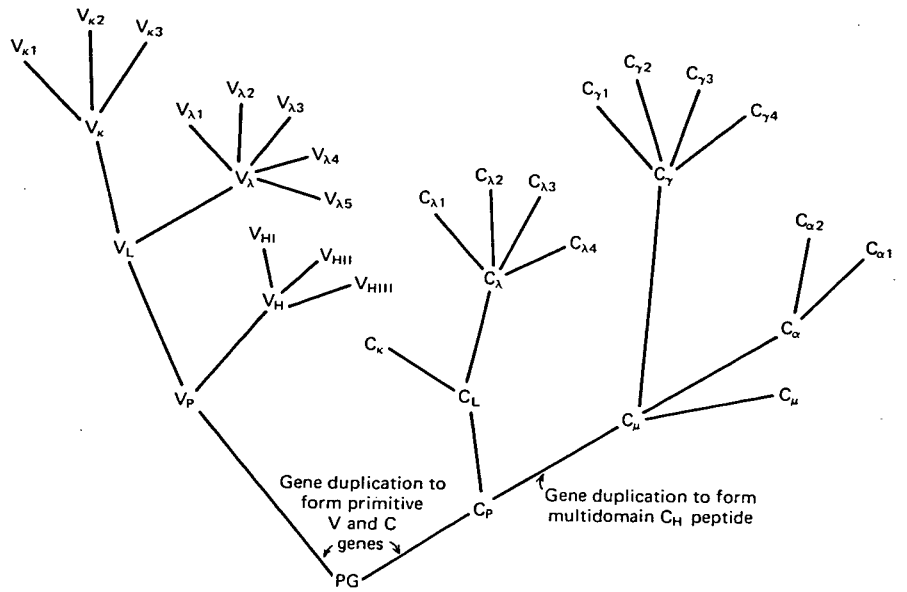

Figure 4-11 Evolution of genes coding for human immunoglobulins. At some point in the distant past, presumably about the time of appearance of the first vertebrates ($4–5 \times 10^8$ years ago) a "precursor gene" (PG) duplicated, and the duplicates diverged to form primitive V and C genes. At this early point of evolution, it is conceivable that a simple VC unit, or a pair of VC units, acted as a primitive sort of antibody molecule. This fairly simple process of gene replication followed by divergent evolution of the new gene copies can easily account for all of the V-region genes, and the various C_L genes. However, at some early point, an important genetic event took place: the "stitching together" of several C_H genes to form coordinately expressed multidomained C_H peptides. The most primitive vertebrates still in existence have multidomained μ-chain-like peptides strikingly similar in overall primary structure to the most recent mammals, suggesting that the "stitching" event must have occurred very early in vertebrate evolution.

globular structures; peptide linkages within each loop are relatively resistant to proteolytic digestion. The intradomain disulfide bonds are much less amenable to reduction by disulfide reagents than are the interchain S–S bonds.

The evolutionary driving force behind development of the multidomained structure for Igs was most likely the addition of useful biological functions with each of the additional domains. The function of the V_H and V_L domains is of course antigen binding. The function of the C_L and C_{H1} domain must certainly be to stabilize the V_L and V_H units so they can interact effectively with antigen. The C_{H2} domain is the site for binding of complement, and either the C_{H3} or the C_{H4} domain (depending on the species and Ig class), appears to be the site for attachment of Ig to cell surfaces (via the so-called Fc receptor). In addition, almost all of

the biological functions distinctive of each Ig class (secretability, passage across the placenta) as well as most of the allotypic determinants, are associated with one or more C_H domains.

The Hinge Region

When the distance between the disulfide loops of each of the H chain domains of IgG, IgD, and IgA are compared, it has been observed that the distance between the C_{H1} and C_{H2} loops is substantially greater than between any other domains. This region, composed of from 12 to 60 additional amino acids depending on the species and H-chain isotype, is referred to as the "hinge" region (Figure 3-5). (IgM and IgE do not have hinge regions.) Hinge regions have been identified in electron micrographs of Ig molecules as a locus of flexibility allowing bending between the Fc and the two Fab arms. The amino acid sequence in this region is highly variable among Ig classes, but, in general, is rich in proline and hydrophilic residues, which render it structurally rather flexible as well as accessible to proteases. This is the region preferentially cleaved by mild papain or pepsin treatment. It also contains most of the interchain disulfide bonds holding the two H chains together.

Fv Fragments

In some instances when Fab fragments are treated with pepsin, Fv fragments, which consist of two adjoining V_H and V_L domains, are obtained. The Fv fragments can retain full antigen-binding capacity. The behavior of these fragments and their component chains (half of an L chain and one-quarter of an H chain) have been studied and have provided important insights into the independency as well as the interdependency of immunoglobulin domains. For example, dissociated Fv fragments can spontaneously recombine in solution to give the correctly folded and fully active dimer. This implies that at least these domains contain within themselves complete information for their own three-dimensional structure. Because antigen binding was also restored, we can conclude that not only can the component chains fold into the appropriate tertiary conformations, but the information for appropriate interaction of the two chains into a quaternary structure must also be contained in the primary sequences of the component domains.

V-Region Fine Structure

We have already alluded to the fact that the NH_2-terminal portions of both H and L chains (V_H and V_L domains) are highly variable in amino acid sequence among members of the same V-region family. (As we will see later, each family [κ, λ, and H] is located on a separate chromosome and contains its own V regions.) However, this heterogeneity is not uniformly distributed within the V_H or V_L region, and

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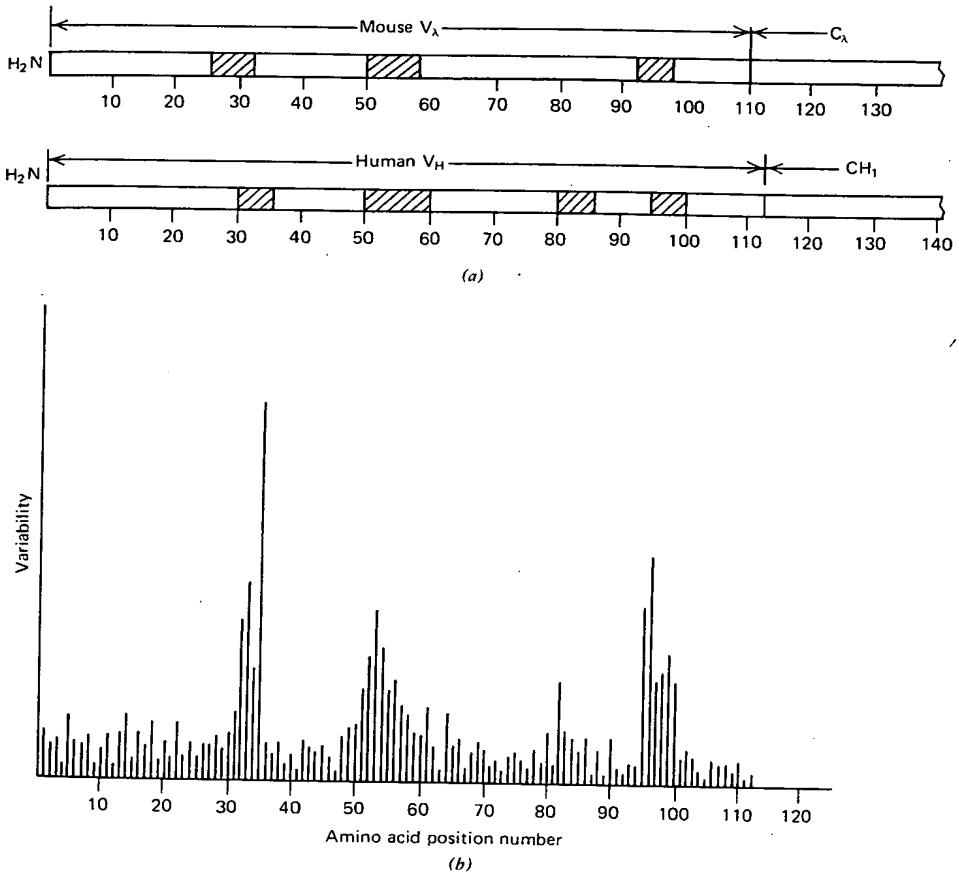

Figure 4-12 (*a*) Detailed structure of the variable region of Ig chains. The first 110 or so amino acid residues at the NH_2-terminal end of both light and heavy chains comprise the variable region. A combination of one V_L and one V_H forms one antigen-binding site. V_H regions generally tend to be slightly longer than V_L by about 5 to 10 amino acids. Not all portions of the variable region are equally variable. The areas of greatest variability, termed the hypervariable regions (hatched regions), are concentrated in discrete locations along the linear array of V-region amino acids. Light chain V regions have three of these so-called "hot spots," whereas V_H regions have four. It is generally accepted that the variability at these amino acid positions is related to variations in antigen-binding capacity. (*b*) Graphic representation of variability devised by Wu and Kabat. The variability at any given amino acid position for the known members of a particular family of V regions can be calculated from the following formula:

$$\text{variability} = \frac{\text{number of different amino acids found at position}}{\text{frequency of most common amino acid at that position}}$$

By simple inspection it will be seen that this function will range from 1, for a completely invariant position, to a maximum of 400 for a position in which all 20 amino acids are found with equal frequency. This particular plot is a composite plot representing an "average" human V_H region.

indeed within each family there are regions of fairly constant amino acid sequences common to all members of the family. The function of these constant regions within the V domains appears to be to maintain the correct peptide conformations for appropriate V–V and V–C interactions, and for correct internal folding of the V region itself.

The variability in V-region amino acid sequences is restricted to three sites in V_κ and V_λ regions in both mouse and humans, to three sites in mouse V_H regions, and to possibly four sites within human V_H regions. By comparing the sequences of a great many myeloma protein V regions within the various families, and analyzing the variability of each amino acid position, Wu and Kabat introduced the concept of "hypervariable regions" (Figure 4-12). These regions are not large, consisting of only about 10 or less amino acids each. All of the variability of the V region is restricted to these sites, and affinity labeling studies (see Chapter 7) have shown that the residues in these regions are the ones that interact with antigenic determinants. As such they can be referred to as "complementarity-determining regions." The amino acid sequences outside the hypervariable regions differ very little within different V-region groups (see definition following of family, group, and subgroup). The overall picture of the V region is thus of a series of "framework" regions (FR), which are relatively invariant, among which are interspersed hypervariable or complementarity-determining regions (CDR). The truly variable CDRs may account for no more than 15 to 20% of total V-region amino acids. The framework regions contain the amino acid residues, important for proper folding of the peptide chains, and for interaction with the corresponding V domain in the opposing chain (*trans* interactions) and with the adjacent C domain on the homologous chain (*cis* interactions). The amino acid residues in the hypervariable regions, as just mentioned, contain the information necessary for binding antigen.

Structure of the Antigen-combining Site

Amino Acids in the Hypervariable Regions Interact with Antigen

The antigen-combining site is formed by noncovalent association of the NH_2-terminal domains of adjacent light and heavy chains. Within each of the two contributing domains are regions of high amino acid variability (the hypervariable or complementarity-determining regions) among members of the same V-region family. A great deal of information has been accumulated to show that the amino acids comprising the hypervariable regions are involved in the binding of antigen. Some of this information has come, and continues to come, from applications of a technique known as affinity labeling.

The principal of affinity labeling is illustrated in Figure 4-13. A chemically reac-

SECOND EDITION

CELLULAR AND MOLECULAR IMMUNOLOGY

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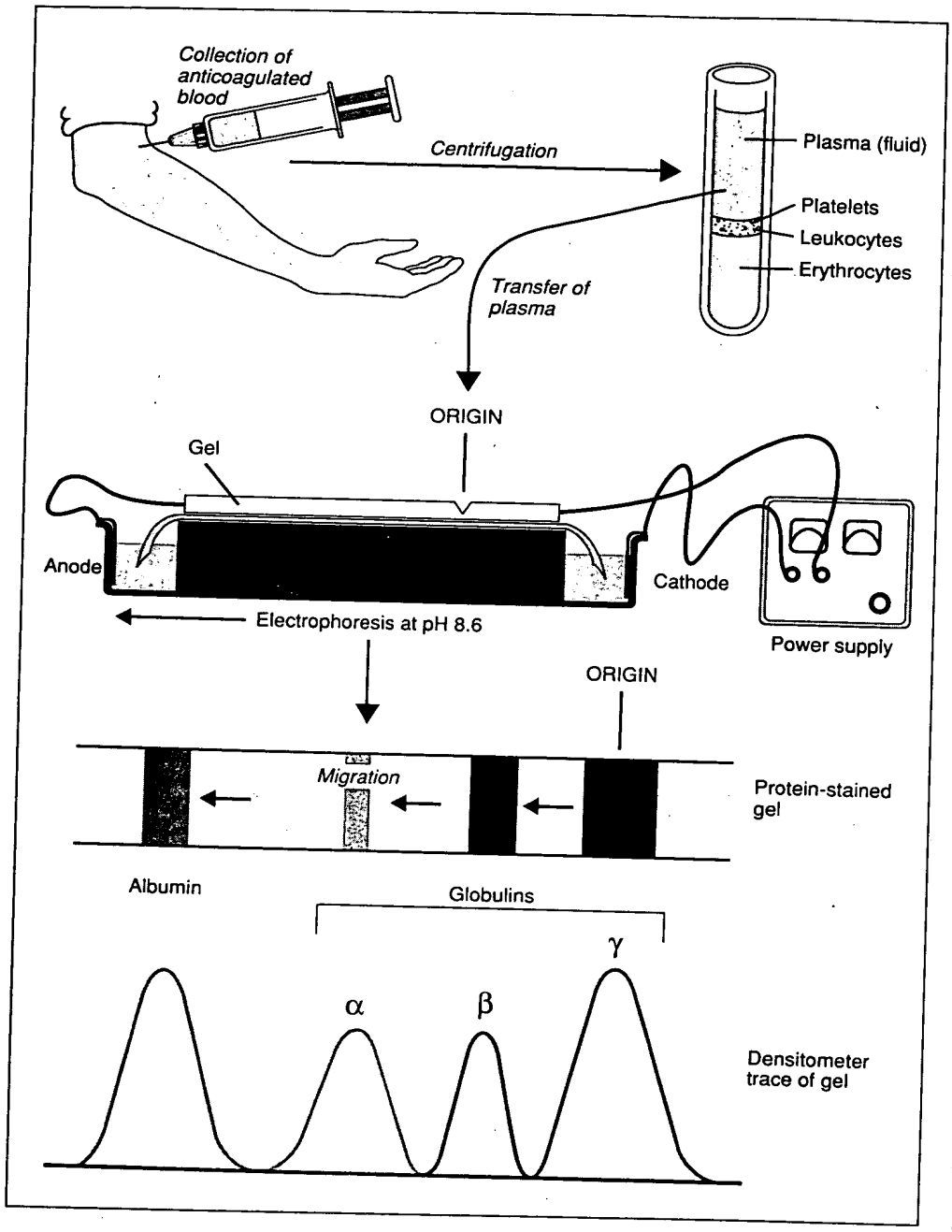

FIGURE 3–1. Separation of plasma proteins by electrophoresis. *Electrophoresis separates plasma proteins into albumin and globulins. Most antibodies are found in the γ globulin fraction.*

rapid and highly resolved separations. This technique is called *high-pressure liquid chromatography* (HPLC).

Overview of Antibody Structure

A number of the structural and functional features of antibodies were determined from the early studies of these molecules:

1. *All antibody molecules are similar in overall structure, accounting for certain common physicochemical features, such as charge and solubility.* These common properties may be exploited as a basis for the purification of antibody molecules from fluids such as blood. *All antibodies have a common core structure of two identical light chains (each about 24 kilodaltons [kD]) and two identical heavy chains (about 55 or 70 kD)* (Fig. 3–2). One light chain is attached to each heavy chain, and the two heavy chains are attached to each other. Both the light chains and the heavy chains contain a series of repeating, homologous units, each about 110 amino acid residues in length, which fold independently in a common globular motif, called an **immunoglobulin domain.** All Ig domains contain two layers of β-pleated sheet with three or four strands of antiparallel polypeptide chain. Certain Ig domains, such as those comprising variable regions (see later), have

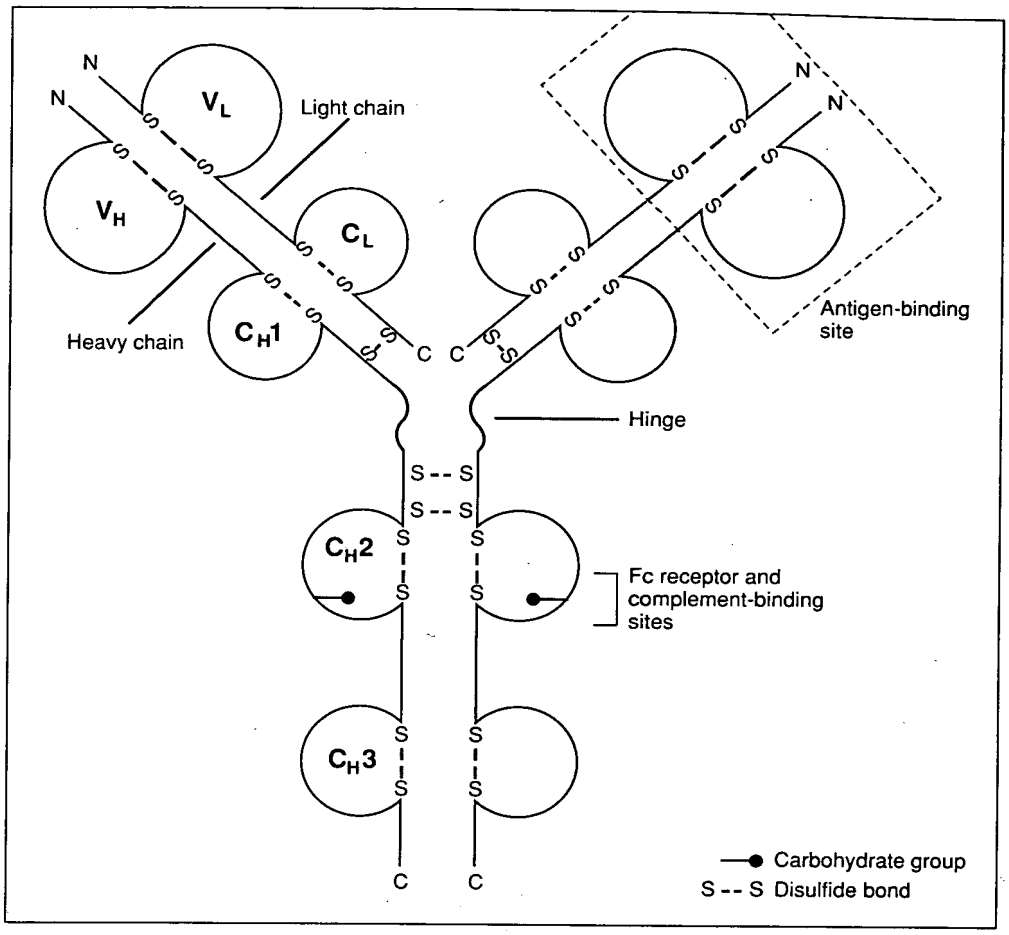

FIGURE 3–2. Schematic diagram of an immunoglobulin molecule. *In this drawing of an IgG molecule, the antigen-binding sites are formed by the juxtaposition of V_L and V_H domains. The locations of complement and Fc receptor–binding sites within the heavy chain constant regions are approximations. S--S refers to intrachain and interchain disulfide bonds; N and C refer to amino and carboxy termini of the polypeptide chains, respectively.*

an extra strand in each of the two layers. As will be discussed in Chapter 7, many other proteins of importance in the immune system contain regions that use the same folding motif and show structural relatedness to Ig amino acid sequences. All molecules that contain this motif are said to belong to the **Ig superfamily,** and all of the gene segments encoding the Ig-like domains are believed to have evolved from the same common ancestral gene (see Chapter 7, Box 7–2).

2. Despite their overall similarity, *antibody molecules can be readily divided into a small number of distinct classes and subclasses, based on minor differences in physicochemical characteristics such as size, charge, and solubility and on their behavior as antigens* (Box 3–2). In humans, the classes of antibody molecules are called IgA, IgD, IgE, IgG, and IgM, and members of each class are said to have the same **isotype** (Table 3–1). IgA and IgG isotypes can be further subdivided into closely related subclasses, or subtypes, called IgA1 and IgA2, and IgG1, IgG2, IgG3, and IgG4, respectively. In certain instances, it will be convenient to refer to studies of mouse antibody. Mice have the same general isotypes as humans, but the IgG isotype is divided into the IgG1, IgG2a, IgG2b, and IgG3 subclasses in mice. The heavy chains of all antibody molecules of an isotype or subtype share extensive regions of amino acid sequence identity but differ from antibodies belonging to other isotypes or subtypes. Heavy chains are designated by the letter of the Greek alphabet corresponding to the overall isotype of the antibody: IgA1 contains $\alpha 1$ heavy chains; IgA2, $\alpha 2$; IgD, δ; IgE, ϵ; IgG1, $\gamma 1$; IgG2, $\gamma 2$; IgG3, $\gamma 3$, IgG4, $\gamma 4$; and IgM, μ. The shared regions of heavy chain amino acid sequences are responsible for both the common physicochemical properties and the common antigenic properties of antibodies of the same isotype. In addition, the shared regions of the heavy chains provide members of each isotype with common abilities to bind to certain cell surface receptors or to other macromolecules like complement and thereby activate particular immune effector functions. Thus, the separation of antibody molecules into isotypes and subtypes on the basis of common structural features also separates antibodies according to which set of effector functions they commonly activate. In other words, *different effector functions of antibodies are mediated by distinct isotypes and subtypes.* As we shall see later, there are two isotypes of antibody light chains, called κ and λ. The light chains, however, do not mediate or influence the effector functions of antibodies.

3. *There are more than 1×10^7, and perhaps as many as 10^9, structurally different antibody molecules in every individual, each with unique amino acid sequences in their antigen-combining sites.* This extraor-

BOX 3–2. ANTI-IMMUNOGLOBULIN ANTIBODIES

Antibody molecules are proteins and therefore can be antigenic. Immunologists have exploited this fact to produce antibodies specific for Ig molecules that can be used as reagents to analyze the structure and function of Ig molecules. In order to obtain an anti-antibody response, it is necessary that the Ig molecules used to immunize an animal be recognized in whole or in part as foreign. The simplest approach is to immunize one species, e.g., rabbit, with Ig molecules of a second species, e.g., mouse. Populations of antibodies generated by such cross-species immunizations are largely specific for epitopes present in the constant regions of light or heavy chains. Such sera can be used to define the isotype of an antibody.

When an animal is immunized with Ig molecules derived from another animal of the same species, the immune response is confined to epitopes of the immunizing Ig that are absent or uncommon on the Ig molecules of the responder animal. Two types of determinants have been defined by this approach. First, determinants may be formed by minor structural differences (polymorphisms) in amino acid sequences located in the conserved portions of Ig molecules. Ig genes that encode such polymorphic structures are inherited as mendelian alleles. (The concepts of polymorphism and allelic genes are discussed more fully in Chapter 5.) Determinants on Ig molecules that differ among animals that have inherited different alleles are called allotopes. All antibody molecules that share a particular allotope are said to belong to the same allotype. Most allotopes are located in the constant regions of light or heavy chains, but some are found in the framework portions of variable regions. Allotypic differences have no functional significance, but they have been important in the study of Ig genetics. For example, allotypes detected by anti-Ig antibodies were initially used to locate the position of Ig genes by linkage analysis. In addition, the remarkable observation that, in homozygous animals, all of the heavy chains of a particular isotype (e.g., IgM) share the same allotype even though the V regions of these antibodies have different amino acid sequences provided the first evidence that the constant portions of all Ig molecules of a particular isotype are encoded by a single gene that is separate from the genes encoding V regions. As will be discussed in Chapter 4, we now know that this surprising conclusion is correct.

The second type of determinant on antibody molecules that can be recognized as foreign by other animals of the same species is that formed largely or entirely by the hypervariable regions of an Ig variable domain. When a homogeneous population of antibody molecules, e.g., a myeloma protein or a monoclonal antibody, is used as an immunogen, antibodies are produced that react with the hypervariable loops. These determinants are recognized as "foreign" because they are usually present in very small quantities in any given animal, i.e., at too low a level to induce self-tolerance (see Chapter 19). Such determinants on individual antibody molecules are idiotopes, and all antibody molecules that share an idiotope are said to belong to the same idiotype. The term idiotype is also used to describe the shared idiotope. As will be discussed in Chapter 4, hypervariable sequences that form idiotopes arise both from inherited germline diversity and from somatic events. Individual idiotopes that arise from somatic events are rare and may define the products of one or a few clones of antibody-producing B cells. Idiotopes that arise from the germline are less rare and, in some cases, may be present on the majority of antibody molecules that recognize a particular antigen (dominant idiotypes). Unlike allotopes, idiotopes may be functionally significant because they may be involved in regulation of B cell functions. The theory of lymphocyte regulation through antibody-binding idiotopes expressed on membrane Ig molecules, called the network hypothesis, is discussed further in Chapter 10.

In addition to experimentally elicited anti-Ig antibodies, immunologists have also been interested in naturally occurring antibodies reactive with self Ig molecules. Small quantities of anti-idiotypic antibodies may be found in normal individuals. Anti-Ig antibodies are particularly prevalent in an autoimmune disease called rheumatoid arthritis (see Chapter 20), in which setting they are known as rheumatoid factor. Rheumatoid factor is usually an IgM antibody that reacts with the constant regions of self IgG. The significance of rheumatoid factor in the pathogenesis of rheumatoid arthritis is unknown.

dinary diversity of structure (whose generation is explained in Chapter 4) accounts for the extraordinary specificity of antibodies for antigens, because each amino acid difference may produce a difference in antigen binding. In theory, such extensive sequence diversity poses a structural problem because the three-dimensional structure of any protein is completely determined by its amino acid sequence and certain sequences are incapable of folding into soluble, stable proteins. In an antibody molecule, this problem is

TABLE 3–1. Human Antibody Isotypes*

Antibody	Subtypes	H Chain (Designation)	H Chain Domains (Number)	Hinge	Tail Piece	Serum Concentration (mg/ml)	Secretory Form	Molecular Weight of Secretory Form (kD)
IgA	IgA1	$\alpha 1$	4	Yes	Yes	3	Monomer, dimer, trimer	150, 300, or 400
	IgA2	$\alpha 2$	4	Yes	Yes	0.5	Monomer, dimer, trimer	150, 300, or 400
IgD	None	δ	4	Yes	Yes	Trace	—	180
IgE	None	ϵ	5	No	No	Trace	Monomer	190
IgG	IgG1	$\gamma 1$	4	Yes	No	9	Monomer	150
	IgG2	$\gamma 2$	4	Yes	No	3	Monomer	150
	IgG3	$\gamma 3$	4	Yes	No	1	Monomer	150
	IgG4	$\gamma 4$	4	Yes	No	0.5	Monomer	150
IgM	None	μ	5	No	Yes	1.5	Pentamer	950

* Multimeric forms of IgA and IgM are associated with J chain via the tail piece region of the heavy chain. IgA in mucus is also associated with secretory piece.

solved by confining the sequence diversity to three short stretches within the amino terminal domains of the heavy and light chains. The amino acid sequences of the amino terminal domains are called **variable (V) regions,** to distinguish them from the more conserved **constant (C) regions** of the remainder of each chain. The highly divergent stretches within the V regions are called **hypervariable regions,** and they are held in place by more conserved **framework regions.** In an intact immunoglobulin, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain can be brought together in three-dimensional space to form an antigen-binding surface. Because these sequences form a surface complementary to the three-dimensional surface of a bound antigen, the hypervariable regions are called **complementarity-determining regions** (CDRs).

With this overview of antibody structure and function in mind, we will now consider antibody structure in greater detail.

Detailed View of Antibody Structure

Light Chain Structure

All antibody light chains fall into one of two classes or isotypes, κ and λ. Each member of a light-chain isotype shares complete amino acid sequence identity of the carboxy terminal C region with all other members of that isotype. In humans, antibodies with κ and λ light chains are present in about equal number. In mice, κ-containing antibodies are about ten times more frequent than λ-containing antibodies. There are no known differences in function between κ-containing and λ-containing antibodies.

Each light chain, whether κ or λ, is folded into separate V and C domains corresponding to the amino terminal and carboxy terminal halves of the polypeptide, respectively (Fig. 3–3). Each domain is about 110 amino acids long. As noted above, most of the amino acid sequence variation among different light chains is confined to three separate locations in the V region. These three hypervariable segments, or CDRs, are each about ten amino acids long (Fig. 3–4). Proceeding from the amino terminus, these regions are the CDR1, CDR2, and CDR3, respectively. CDR3 is the most variable of the CDRs, and, as will be discussed in Chapter 4, there are more genetic mechanisms for generating sequence diversity in this region than in CDR1 and CDR2. V region folding into an Ig domain is mostly determined by the sequence of the framework regions adjacent to the CDRs. Within the framework regions, certain amino residues and certain structural features are very highly conserved. For example, all V region sequences contain an internal disulfide loop of about 90 amino acid residues. Other portions of the framework regions differ between κ and λ chains. When V_κ or V_λ regions fold into an Ig domain, the CDRs are present on the surface as projecting loops (Fig. 3–3). Recent studies suggest that each CDR (except CDR3 of the heavy chain) folds similarly, regardless of the precise amino acid sequence, suggesting that there are conserved ("canonical") structural features within the hypervariable segments of antibodies. Sequence differences among the CDRs of different antibody molecules result in unique chemical structures being projected at the surfaces of the projecting loops. As we shall discuss shortly, these *variations in surface structure account for specificity for antigens.*

The carboxy terminus of the C region of the light chain also folds into an Ig domain. Although C_κ and C_λ differ in exact amino acid sequence, they are structurally related, or homologous, to each other and, to a lesser extent, to V_κ and V_λ.

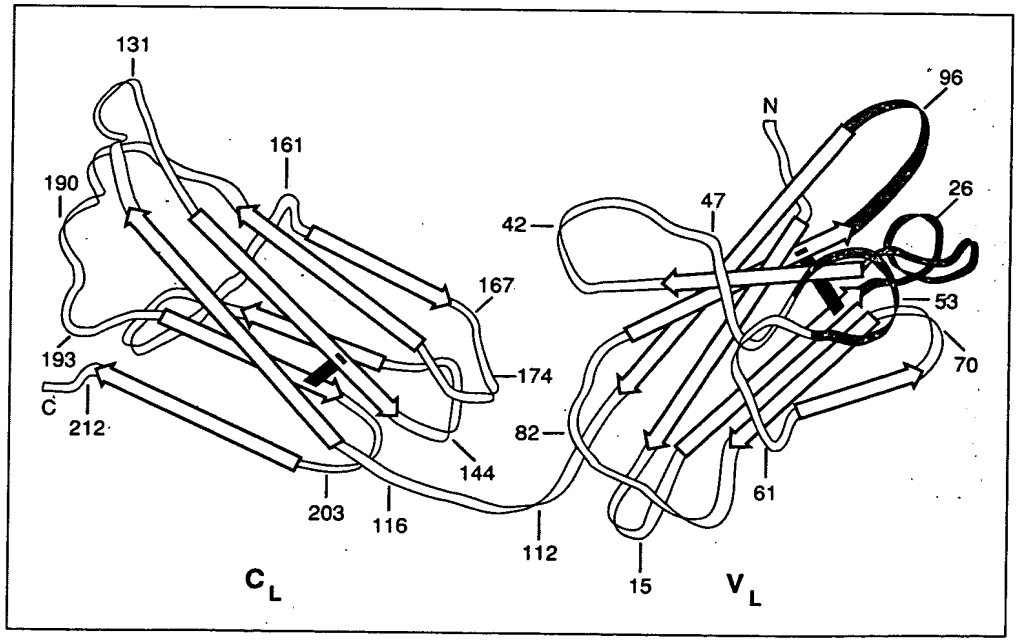

FIGURE 3–3. Polypeptide folding into Ig domains in a human antibody light chain. *The V and C regions each independently fold into Ig domains. The white arrows represent polypeptide arranged in β-pleated sheets, the dark blue bars are intrachain disulfide bonds, and the numbers indicate the positions of amino acid residues counting from the amino (N) terminus. The CDR1, CDR2, and CDR3 loops of the V region, colored in light blue, are brought together to form the antigen-binding surface of the light chain. (Adapted with permission from Edmundson, A. B., K. R. Ely, E. E. Abola, M. Schiffer, and N. Panagiotopoulos. Rotational allostery and divergent evolution of domains in immunoglobulin light chains. Biochemistry 14:3953–3961, 1975. Copyright 1975, American Chemical Society.)*

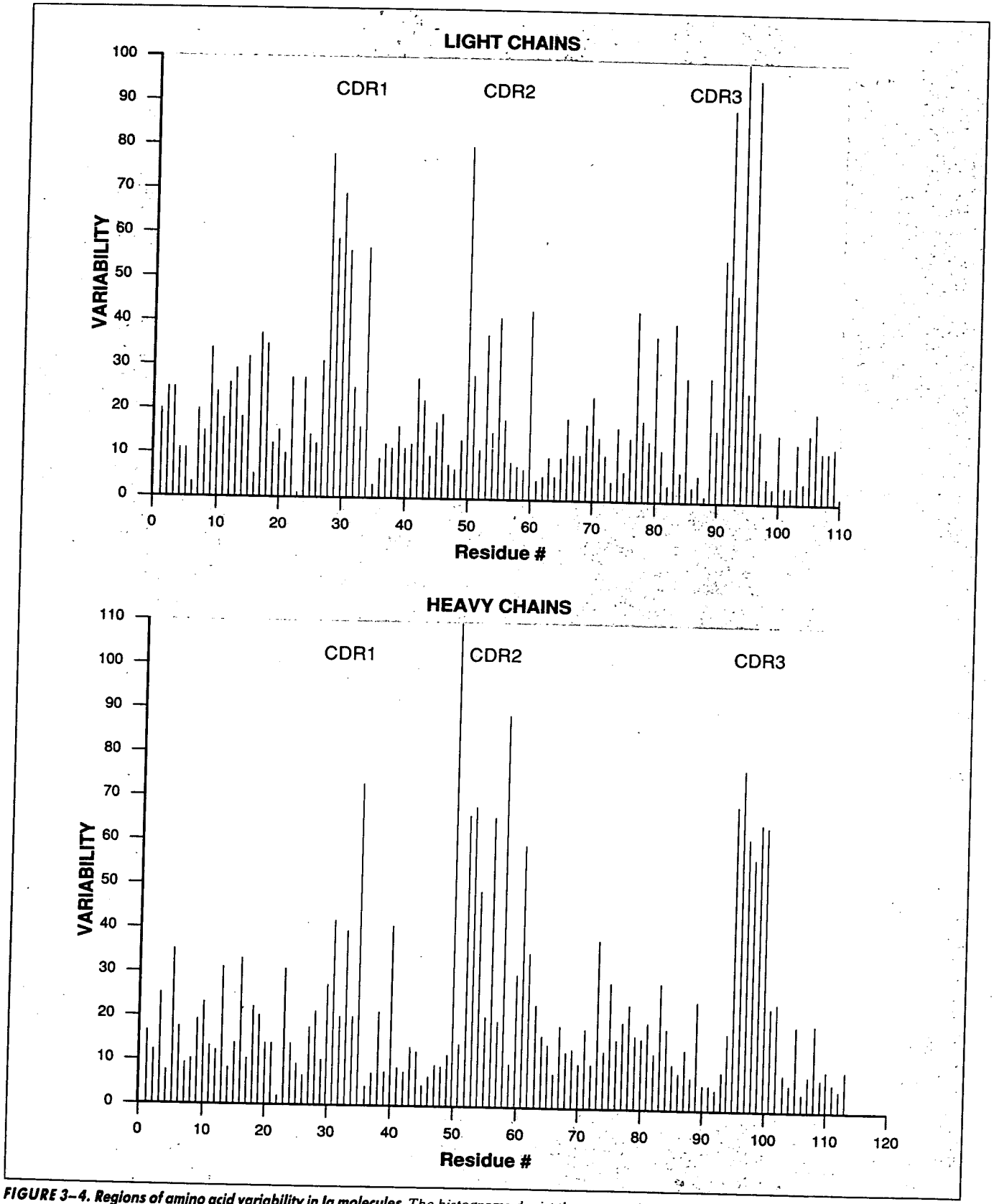

FIGURE 3–4. Regions of amino acid variability in Ig molecules. *The histograms depict the extent of variability, defined as the number of differences in each amino acid residue among various independently sequenced Ig heavy and light chains, plotted against amino acid residue number, measured from the amino terminus. This method of analysis, developed by Elvin Kabat and Tai Te Wu, indicates that the most variable residues are clustered in three "hypervariable" regions, colored in blue, corresponding to the three CDRs shown in Figure 3–3. (Courtesy of Dr. E. A. Kabat, Department of Microbiology, Columbia University College of Physicians and Surgeons, New York.)*

HEAVY CHAIN STRUCTURE

All heavy chain polypeptides, regardless of antibody isotype, contain a tandem series of segments, each approximately 110 amino acid residues in length. These segments are homologous to each other, and all undergo characteristic folding into 12 kD Ig domains. As in light chains, the amino terminal variable, or V_H, domain displays the greatest sequence variation among heavy chains, and the most variable residues are concentrated into three short (up to ten amino acid residue) stretches called CDR1, CDR2, and CDR3 (Fig. 3–4). Also similar to light chains, the heavy chain CDR3 shows greater variability in sequence and folding pattern than CDR1 or CDR2.

The remainder of the heavy chain, which forms the constant (C) region, differs among isotypes; however, it is invariant among the member antibodies within a particular isotype. In IgM and IgE antibodies, the constant region folds to form four tandem Ig domains. In IgG, IgA, and IgD antibodies, the shorter constant regions form three Ig domains. (In the mouse, the δ chain gene has undergone a deletion such that the constant region forms only two Ig domains.)

In γ, α, and δ heavy chains, there is a nonglobular segment, containing from about ten (in $\alpha 1$, $\alpha 2$, $\gamma 1$, $\gamma 2$, and $\gamma 4$) to over 60 (in $\gamma 3$ and δ) amino acid residues, located between the first and second constant region domains (called C_H1 and C_H2, respectively). Although portions of this sequence form rodlike helical structures, other portions assume a random and flexible conformation, permitting molecular motion between C_H1 and C_H2. For this reason, this segment of the heavy chain is called the **hinge.** Some of the greatest differences between the constant regions of the IgG subclasses are concentrated in the hinge. For steric reasons, antibody subtypes with flexible hinges may be better able to use more than one antigen-binding site to attach to a particular antigen; as discussed later in this chapter, binding involving more than one attachment point will increase the strength of attachment.

All heavy chains may be expressed in one of two molecular forms that differ in amino acid sequence on the carboxy terminal side of the last C_H domain. The secretory form, found in blood plasma, terminates with a sequence containing charged and hydrophilic amino acid residues. The membrane form, found only on the plasma membrane of the B lymphocyte that synthesized the antibody, has distinct carboxy terminal sequences that include approximately 26 uncharged, hydrophobic side chains followed by variable numbers of charged (usually basic) amino acid residues that form the cytoplasmic segments (Fig. 3–5). This structural motif is characteristic of transmembrane proteins. The hydrophobic residues are believed to form an α-helix, which extends across the hydrophobic portion of the membrane lipid bilayer; the basic side chains of the cytoplasmic amino acids interact with the phospholipid head groups on the cytoplasmic surface of the membrane. In membrane IgM or IgD, the extreme carboxy terminus or cytoplasmic portion of the heavy chain is very short, only three amino acid residues; in membrane IgG or IgE, it is somewhat longer, up to about 30 amino acid residues in length.

The secretory forms of μ, α, and δ heavy chains, but not γ or ϵ, have additional extended nonglobular sequences on the carboxyterminal side of the last C_H domain. These extensions are called **tail pieces.** In secreted IgM and IgA molecules, the tail pieces contribute toward intermolecular interactions that result in the formation of multimeric Ig molecules. Specifically, IgM forms a pentamer, containing ten heavy chains and ten light chains, and IgA can form dimers containing four heavy chains and four light chains, or trimers, containing six heavy chains and six light chains (Fig. 3–6). Little is known about the usual form of circulating IgD because it is normally present in only trace amounts in the blood. Multimeric IgM and IgA also contain an additional 15 kD polypeptide, called the **joining (J) chain,** which is disulfide-bonded to the tail pieces, stabilizing the multimer. All membrane Ig molecules, regardless of isotype, are believed to be monomeric, containing two heavy and two light chains.

All heavy chains are characteristically N-glycosylated; that is, the polypeptide contains N-linked oligosaccharide groups attached to asparagine side chains. The location of oligosaccharides may vary in different Ig isotypes. The precise composition of the oligosaccharides is not fully determined by the polypeptide sequence and may also vary with the physiologic state of the host at the time of antibody synthesis.

ASSOCIATION OF LIGHT AND HEAVY CHAINS

The basic pattern of chain association in all antibody molecules is that each light chain is attached to a heavy chain and each heavy chain pairs with another heavy chain. The association between light and heavy chains involves both covalent and non-covalent interactions (see Fig. 3–2). Covalent interactions are in the form of disulfide bonds between the carboxy terminus of the light chain and the C_H1 domain of the heavy chain. The exact position of the heavy chain cysteine that participates in disulfide bond formation varies with the isotype. Non-covalent interactions arise primarily from hydrophobic interactions between V_L and V_H domains and between the C_L domain and the C_H1 domain. This association of V_L and V_H domains produces a spatial apposition such that the juxtaposed V domains can each contribute to the binding of antigen (see Plate I, opposite page 50).

The pairing of heavy chains is best understood from studies of IgG molecules. As in the case of light and heavy chain association, both covalent and noncovalent interactions are involved. Heavy chains form interchain disulfide bonds in the region near the carboxy terminus of the hinge. Extensive non-covalent interactions occur between the C_H3 domains. In contrast, there is little favorable interaction between the polypeptides of the C_H2 domains. Some of the N-linked oligosaccharides are located in a physical gap formed between these portions of the chain and may positively interact with each other, contributing to interchain associations. The length and flexibility of the hinge re-

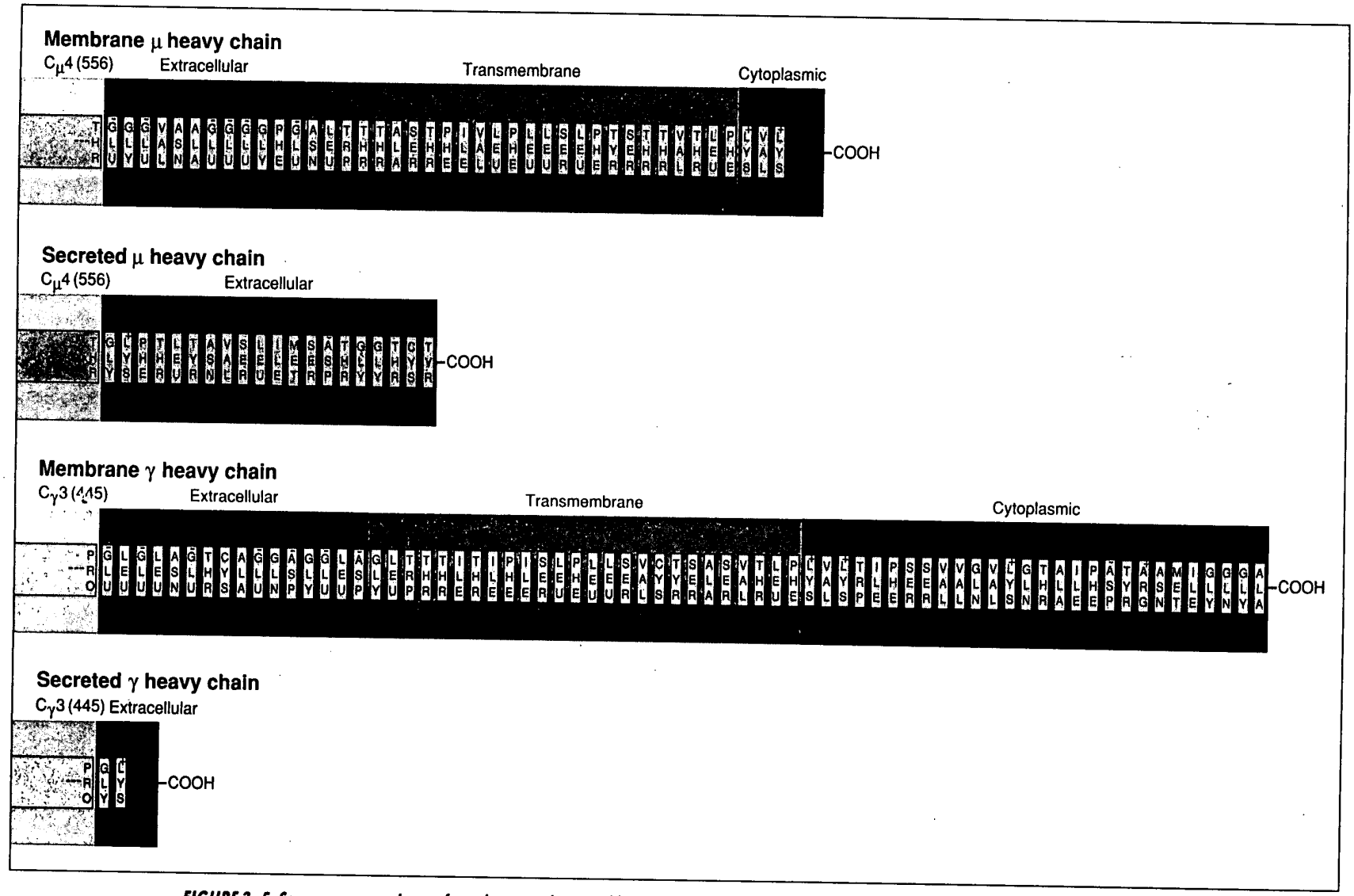

FIGURE 3–5. Sequence comparisons of membrane and secreted forms of Ig heavy chains. *Membrane forms of Ig heavy chains contain characteristic hydrophobic sequences, the transmembrane region, which span the lipid bilayer of the plasma membrane. The cytoplasmic domains of membrane heavy chains of different isotypes are significantly different: μ contains only three residues, whereas γ3 contains 28. The carboxy termini of secreted forms also differ among isotypes: μ has a long tail piece involved in pentamer formation, whereas γ3 does not. Amino acids are shown in the three-letter code, and charged residues are marked + or −; the numbers in parentheses mark the amino acid residue number of the carboxy terminus of the last Ig domain (i.e., $C_\mu 4$ or $C_\gamma 3$).*

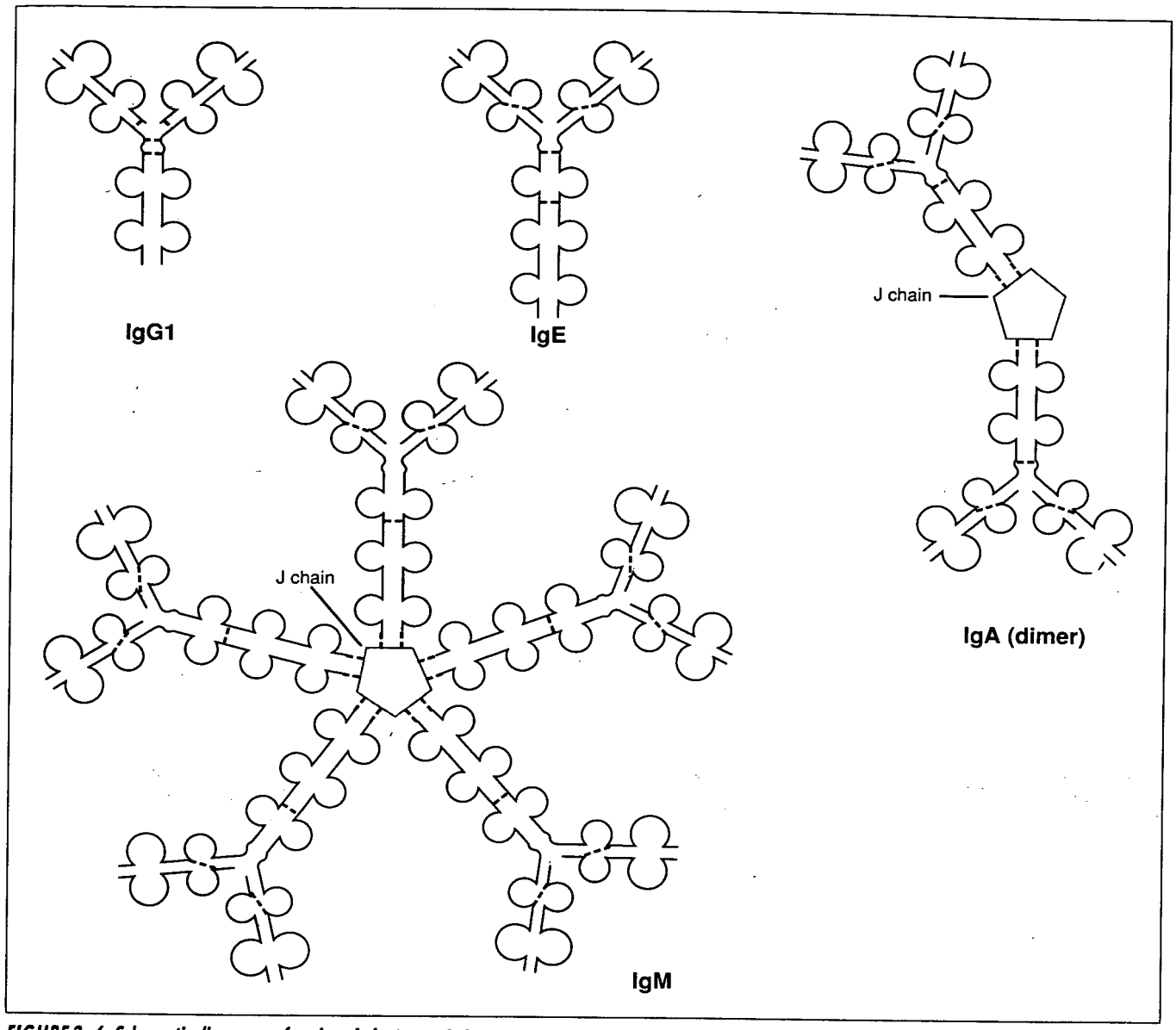

FIGURE 3–6. Schematic diagrams of various Ig isotypes. *IgG and IgE circulate as monomers, whereas secreted forms of IgA and IgM are dimers and pentamers, respectively, stabilized by the J chain. (Some IgA molecules are trimers, not shown.)*

gions differ significantly among IgG subclasses because, as noted above, most of the amino acid sequence differences among the four subclasses are located in the hinge region. These sequence differences lead to very different overall shapes among the IgG subtypes, as depicted in Figure 3–7.

These structural features of chain association explain the results of the classical limited proteolysis studies of rabbit IgG conducted by Rodney Porter and colleagues. The theory of limited proteolysis is that globular or rodlike domains of folded proteins are more resistant to the peptide-bond cleaving actions of proteolytic enzymes than are extended, flexible regions of polypeptide. In IgG molecules, the most susceptible region is therefore the hinge located between $C_\gamma1$ and $C_\gamma2$ of the heavy chain. The proteolytic enzyme papain preferentially cleaves rabbit IgG molecules into three separate pieces (Fig. 3–8). Two of the pieces are identical to each other and consist of an intact light chain associated with a V_H–$C_\gamma1$ fragment of the heavy chain. These fragments each retain the ability to bind antigen, a function of the V_L and V_H domains, and are therefore called **Fab** (fragment, antigen-binding). The third piece contains identical fragments of the γ heavy chain composed of the $C_\gamma2$ and $C_\gamma3$ domains. This piece of IgG has a propensity to self-associate and to crystallize into a lattice. It is therefore called **Fc** (fragment, crystalline). Lattice formation depends upon a uniformity of structure. The propensity of Fc regions to form a lattice reflects the presence of common amino acid sequences of the $C_\gamma2$ and $C_\gamma3$ domains shared by all antibodies of the same subtype. As we shall discuss later in this chap-

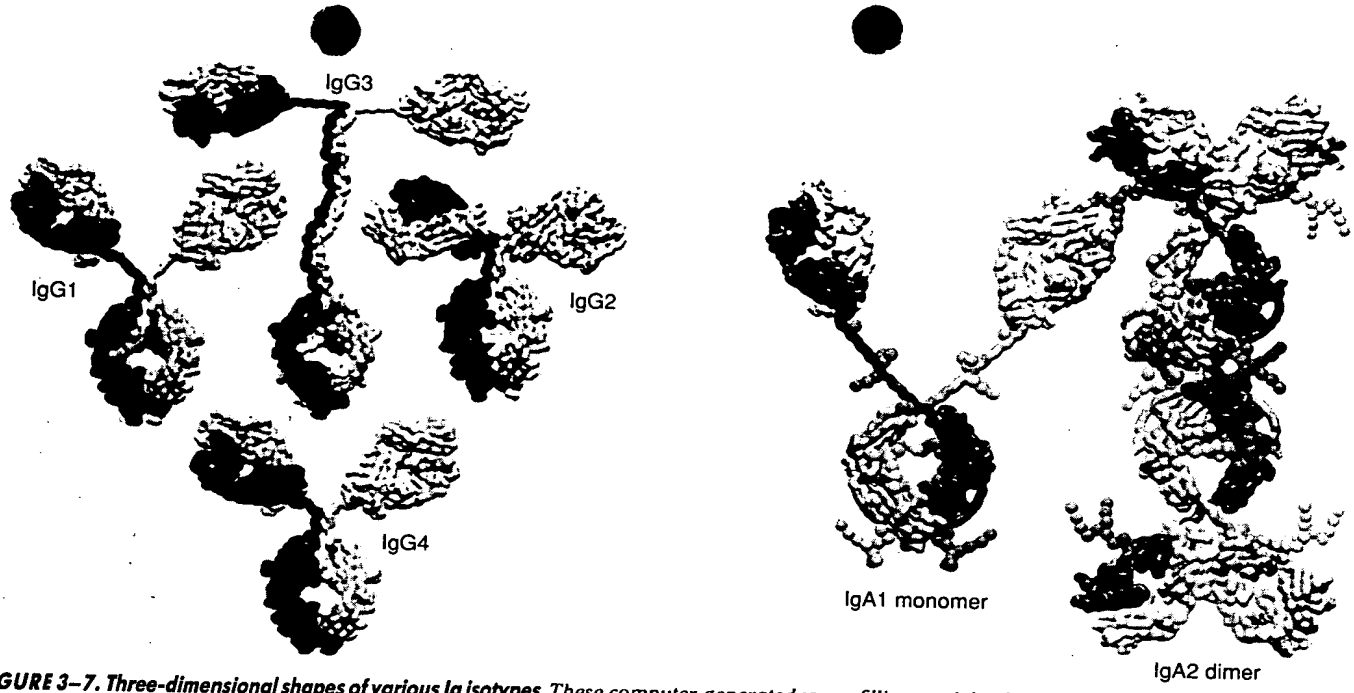

FIGURE 3–7. Three-dimensional shapes of various Ig isotypes. *These computer-generated space-filling models of different Ig isotypes illustrate that the shapes of antibody molecules are quite distinct, largely owing to differences in the lengths of the hinge regions. (Courtesy of Dr. R. S. H. Pumphrey, Regional Immunology Service, St. Mary's Hospital, Manchester.)*

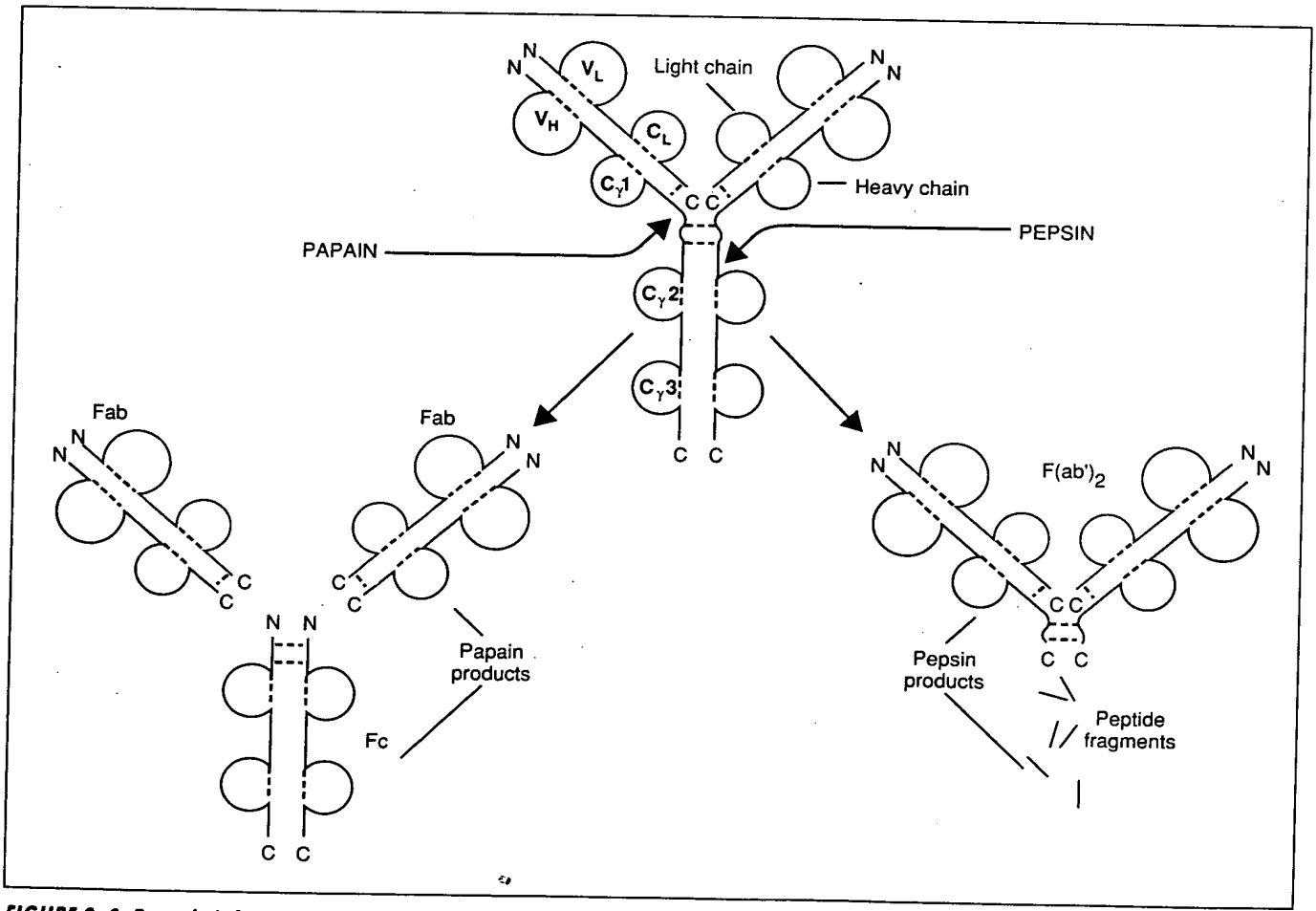

FIGURE 3–8. Proteolytic fragments of an IgG molecule. *Sites of papain and pepsin cleavage are indicated by arrows. Papain digestion allows separation of two antigen-binding regions (the Fab fragments) from the portion of the IgG molecule that activates complement and binds to Fc receptors (the Fc fragment). Pepsin generates a single bivalent antigen-binding fragment [$F(ab')_2$] with higher avidity for antigen than the two monovalent Fab fragments produced by papain cleavage.*

ter, many of the effector functions of immunoglobulins are mediated by the Fc portions of the molecule.

Different results are obtained when the proteolytic enzyme pepsin is used instead of papain to cleave rabbit IgG molecules (Fig. 3–8). In this case, under limiting conditions of enzyme concentrations and time, proteolysis is restricted to the carboxy terminus of the hinge region near the $C_\gamma 2$ domain such that the antigen-binding fragment of IgG retains the hinge and the interchain disulfide bonds. Fab fragments containing the heavy chain hinge are called Fab′; when the interchain disulfide bonds are intact, the two Fab′ fragments remain associated in a form called **F(ab′)$_2$.** The Fc fragment is often extensively degraded and does not survive proteolysis by pepsin. Fab and F(ab′)$_2$ are often useful as experimental tools because they can bind to antigens without activating Fc-dependent effector mechanisms.

These proteolysis experiments are not readily extended to other antibody isotypes such as IgM. In fact, they are not even applicable to all IgG molecules in many species other than rabbit. However, the basic organization of the Ig molecule that Porter deduced from his studies of rabbit IgG is common to all Ig molecules of all isotypes and of all species. These features may be summarized as follows:

1. Each V_LV_H pairing forms an independent antigen-binding site. Thus, all monomeric IgG molecules have two separate antigen-binding sites, and secreted pentameric IgM molecules have ten separate antigen-binding sites (see Figs. 3–2 and 3–6).
2. The structure of the hinge region (or lack of one in certain isotypes) sterically determines how many binding sites of a single antibody molecule can simultaneously interact with antigen molecules, e.g., on a cell surface.
3. The Fc portion of an antibody molecule is spatially distinct from and functions independently of the antigen-binding site formed by the Fab regions. Since Fc regions activate immune effector functions, the kinds of effector functions activated by a particular Ig molecule are largely independent of the specificity for antigen and instead depend primarily on the isotype of the antibody.

ANTIBODY BINDING OF ANTIGENS

In the preceding sections, we have developed a general description of the structure of antibody molecules. Now we will turn to a more detailed discussion of the structural basis and physicochemical characteristics of antigen binding.

Structural Aspects of Biologic Antigens

An **antigen** can be defined as any substance that may be specifically bound by an antibody molecule. This differs from the original (historical) definition of antigen as a molecule that generates an antibody. We now know that almost every kind of biologic molecule, including simple intermediary metabolites, sugars, lipids, autacoids, and hormones as well as macromolecules such as complex carbohydrates, phospholipids, nucleic acids, and proteins, can serve as antigens. However, only macromolecules can initiate lymphocyte activation necessary for an antibody response. Molecules that generate immune responses are called **immunogens.** (Although technically less precise, the more inclusive term "antigens" is still commonly used to refer to "immunogens.") In order to generate antibodies specific for small molecules, immunologists commonly attach such small molecules to macromolecules before immunization. In this system, the small molecule is called a **hapten** and the macromolecule, usually a foreign protein, is called a **carrier.** The hapten-carrier complex, unlike free hapten, can act as an immunogen.

In general, macromolecules are much bigger than the antigen-binding region of an antibody molecule. Therefore, an antibody binds to only a specific portion of the macromolecule, called a **determinant,** or **epitope.** These two words are synonymous and are used interchangeably throughout the book. A hapten may be thought of as an exogenous determinant that is attached to a macromolecule.

Macromolecules typically contain multiple determinants, each of which, by definition, can be bound by an antibody. In some cases, the determinants are spatially well separated, and two individual antibody molecules can be bound to the same antigen molecule without influencing each other; such determinants are said to be non-overlapping. In other cases, the first antibody bound to an antigen may sterically interfere with the binding of the second, and the determinants of the antigen are said to be overlapping. In rarer cases, binding of the first antibody may cause a conformational change in the structure of the antigen, influencing the binding of the second antibody by means other than steric hindrance. Such interactions are called allosteric effects.

In the case of phospholipids or of complex carbohydrates, the antigenic determinants are entirely a function of the covalent structure of the macromolecule. However, in the case of nucleic acids, and even more so in the case of proteins, the non-covalent folding of the macromolecule may also contribute to the formation of determinants. In proteins, epitopes formed by adjacent amino acid residues in the covalent sequence are called **linear determinants** (Fig. 3–9). It is estimated that, in a protein antigen, the size of the linear determinant that forms contacts with specific antibody is about six amino acids long. Linear determinants may be accessible to antibodies in the native folded protein if they appear on the surface or in a region of extended conformation. More often, linear determinants may be inaccessible in the native conformation and appear only when the protein is denatured. In contrast, **conformational determinants** are formed by amino acid residues from separated portions of the linear amino acid sequence that are spatially juxtaposed only upon folding (Fig. 3–9). In theory, denatured proteins could transiently give rise to conformational